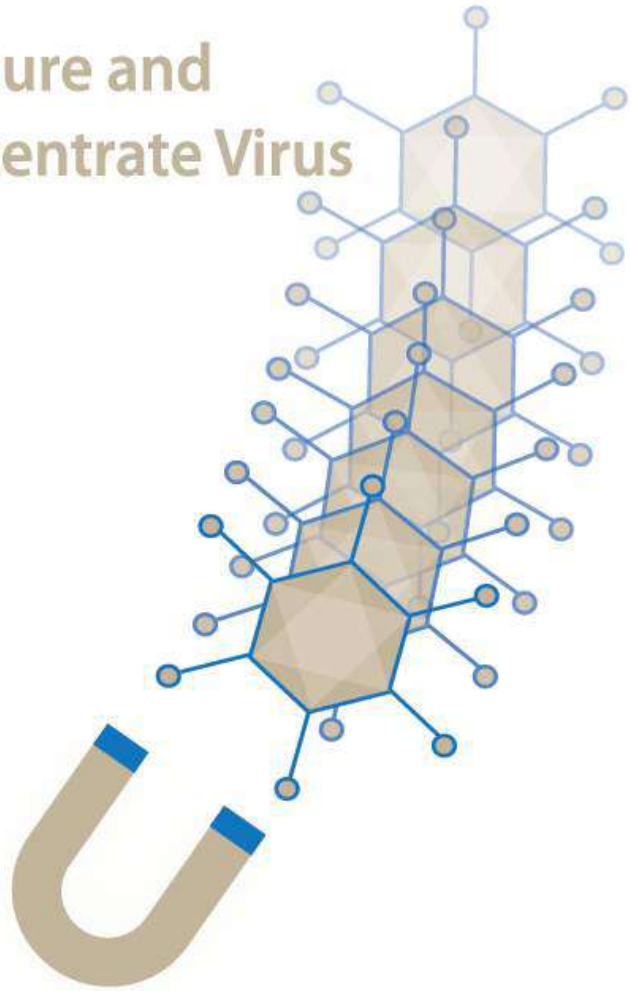
Mag4C™ - Lv Kit

INSTRUCTION MANUAL

Capture and concentrate Virus



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Mag4C-Lv Kit

Instruction Manual

Mag4C-Lv Kit for magnetic capture, concentration and conservation of Lentiviruses and Retroviruses

List of Mag4C-Lv Kits

Catalog Number	Description	Contents	Number of Captures*
LTK11200	Mag4C-Lv Trial kit	✓ Mag4C-Lv beads (0.2 mL) ✓ Elution Buffer (5 mL) ✓ Conservation Buffer (0.2 mL)	Up to 20
LKC11000	Mag4C-Lv kit	✓ Mag4C-Lv beads (1mL) ✓ Elution Buffer (5mL) ✓ Conservation Buffer (1mL)	Up to 100
MSR1000	Magnetic Separation rack	✓ Magnetic Separation rack	NA

* Number of captures based on 1 mL of virus preparation.

Table of Contents

1. Technology

1.1. Description

Mag4C-Lv Kit is specifically designed and developed for capturing, concentrating and storing Lentiviruses and Retroviruses. This kit is composed of 3 reagents allowing **Magnetic Capture/Concentration, Elution and Conservation** of Lentiviruses/Retroviruses.

Mag4C-Lv magnetic nanoparticles capture by electrostatic and hydrophobic interactions viruses in culture medium with 80-99 % efficiency. Once captured onto magnetic beads, viruses can be:

(1) Concentrated and stored with the **Conversation Buffer** or directly used for cell culture, molecular biology or other assays.

(2) Concentrated, eluted from the magnetic beads with the **Elution Buffer** and stored with the **Conversation Buffer** or used for various assays.

The **Conservation Buffer** has been expressly designed to improve the stability of Lentiviruses/Retroviruses upon storage conditions. This buffer is fully compatible with magnetic nanoparticles, meaning that virus bound to magnetic beads can be diluted directly into the buffer for long term storage.

Mag4C-Lv Kit is dedicated to Lentiviruses/Retroviruses and presents unique properties:

For concentration

1. Concentration of viruses by magnetic capture in 30-45 minutes
2. Simple, rapid & ready-to-use: No need to process magnetic beads before capture
3. High yield of viral capture and recovery
4. Fast concentration (2 to 1000 X)
5. Avoid ultracentrifugation, precipitation and chemicals: no stringent buffer or physical action on viruses
6. Reduced handling steps of viruses (minimized bio-hazard)
7. Suitable for large volumes
8. Serum compatible & Non Toxic
9. Ideal for cell culture transduction/infection (Magnetofection™ advantages)

For conservation

1. Improved virus preservation upon storage (-80°C)
2. Maintain high virus titers upon freeze and thaw cycle
3. Compatible with magnetic nanoparticles

1.2. Kit Contents

OZ Biosciences offers two sizes of Mag4C-Lv Kit (trial and kit). **Mag4C-Lv Kit contents**:

- 1 tube containing 0.2 mL (trial) or 1 mL of Mag4C-Lv beads
- 1 tube containing 5 mL of Elution Buffer (1X).
- 1 tube containing 0.2 mL (trial) or 1 mL of Conservation Buffer (5X).

The kit do not contain the Magnetic Separation Rack that must be purchased separately

Stability and Storage

- Mag4C-Lv beads, Elution Buffer Storage and Conservation Buffer Storage: +4°C.
- Magnetic Separation Rack can be stored at room temperature

Kit components are stable for at least one year at the recommended storage temperature.

- *Do not freeze the Mag4C-Lv beads!*
- *Do not add anything to the Mag4C-Lv beads and buffers!*

Shipping condition: Room Temperature

2. Applications

2.1. Virus Types

Mag4C-Lv beads can be combined with any Lentiviruses/Retroviruses.

2.2. Downstream Biological Assays

After magnetic capture and concentration, viruses can be used for multiple assays. For instance, they can be used for PCR, western blot, ELISA, *in vitro* and *in vivo* infection, etc. Viruses can be directly used with the bound Mag4C-Lv beads or eluted from the Mag4C-Lv beads (free of beads). For cell biology, we suggest to use viruses associated with Mag4C-Lv beads to infect cells. Virus complexed to Mag4C-Lv beads, eluted virus (free of beads), and virus in conservation buffer have all been successfully tested on a variety of immortalized and primary cells.

For in vitro and in vivo infection. Mag4C-Lv beads are compatible with the Magnetofection™ technology. This method allows concentrating the entire viral dose on the cells very rapidly, accelerating the transduction process and infecting non-permissive cells. Moreover, virus infection efficiency is significantly increased and cell adsorption/infection can be synchronized without modification of the viruses. Targeted/confined transduction to specific area (magnetic targeting) can also be accomplished.

3. Magnetic separation rack and Magnetofection™ apparatus

Mag4C-Lv Kit requires appropriate magnetic fields for concentrating magnetized viruses.

The Magnetic Separation Rack (#MSR1000, photo), is designed for 50, 15 or 1.5 mL tubes. It can hold 12 standard microtubes, two 15 mL and two 50 mL tubes. The Magnetic Separation Rack is required for capture, concentration, washing and elution.

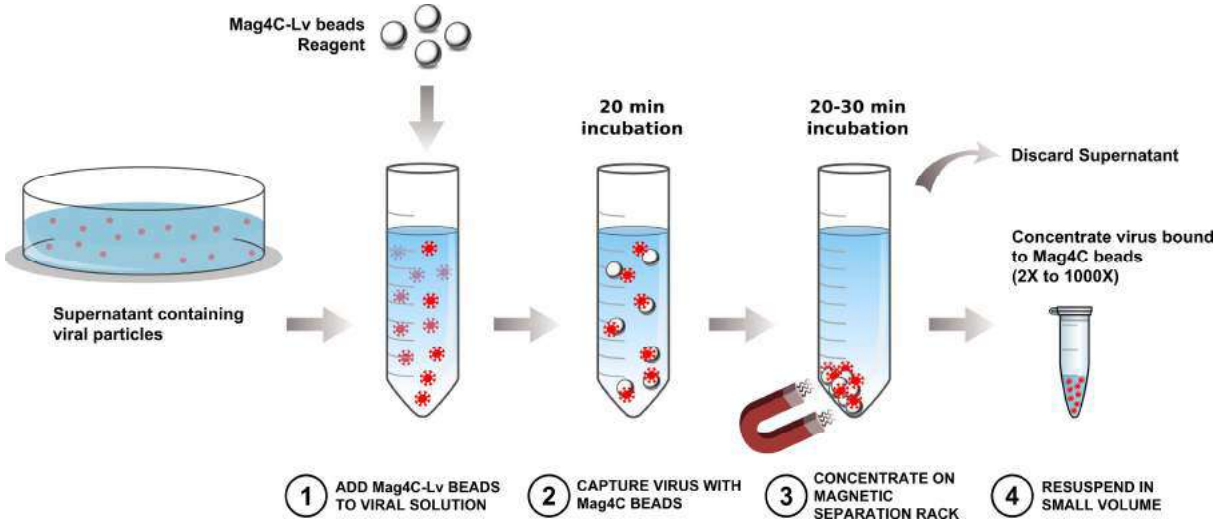
If Mag4C-Lv beads are kept bound to viruses and to take advantage of the Magnetofection™ technology for the downstream cell culture or *in vivo* assays, a magnetic plate or magnets are needed. OZ Biosciences provide specific magnetic plates for Magnetofection™: 96-magnets plate (#MF10096), super magnetic plate (#MF10000) and mega magnetic plate (#MF14000) and specific magnets set for *in vivo* applications (#IV-MAG1).

4. Protocol

4.1. General Considerations

The instructions given below represent typical protocols that were applied successfully to **Capture**, **Concentrate, Elute**, and **Conserve** freshly produced or purchased viruses. Our R&D team has tested and optimized the **Mag4C-Lv Kit** in order to provide you with the most straightforward and efficient procedure. Therefore, we suggest you to start by following our general protocol as guidelines to obtain good data rapidly. Thereafter, we recommend optimizing the conditions to achieve the best performance. Indeed, optimal conditions vary from one virus production to another and are highly dependent upon the type of virus used, its titer, the composition of the viral solution, and cell culture conditions. We advise you to optimize the experimental condition parameters as described in the Appendix in order to achieve the best effects.

4.2. Capture/Concentration Protocol



The protocol is simple: 20 µL of **Mag4C-Lv** beads are sufficient to bind $1x10^6$ infectious viruses with almost 80-99% efficiency. Please refer to the **Table 1** for the suggested **Mag4C-Lv** beads volume according to the virus titer.

Depending on the virus type, the total virus quantity (particles and infectious), and the complexity of the medium, this protocol would have to be adjusted (see appendix). It is recommended to raise the volume of **Mag4C-Lv** beads for complex medium (complete culture medium, organic fluids...).

Table 1 Recommended volume of **Mag4C-Lv** beads according to the number of infectious viruses

Viral Preparation (mL)	Mag4C-Lv beads (µL) "Starting Point" *	Mag4C-Lv beads (µL) Suggested range of testing
≤ 2 mL	20 µL	10 µL – 40 µL
> 2mL	10 µL / mL	5 µL – 20 µL / mL

* for high titer viral solution (≥ 10^7 infectious viruses / mL), we recommend using 1.5 or 2 times the suggested volume.

Important: The suggested volume of Mag4C-Lv beads for capture is related to infectious particles and not physical viral particles.

1) Add 20 µL of **Mag4C-Lv** beads into the virus preparation ≤ 2 mL or 10 µL / mL of **Mag4C-Lv** beads for virus preparation > 2 mL. It may be necessary to adjust the volume of Mag4C-Lv depending on the composition of the virus solution (see Table 1).

2) Incubate 20-30 min at room temperature to capture viruses.

3) Place the tube 15 to 30 min onto the Magnetic Separation Rack to concentrate the virus/Mag4C-Lv beads complexes. Incubation time will depend on the tube volume (see Table 2). Then, discard supernatant.

NOTE: Brown pellet should be visible on the side of the tube near the magnets.

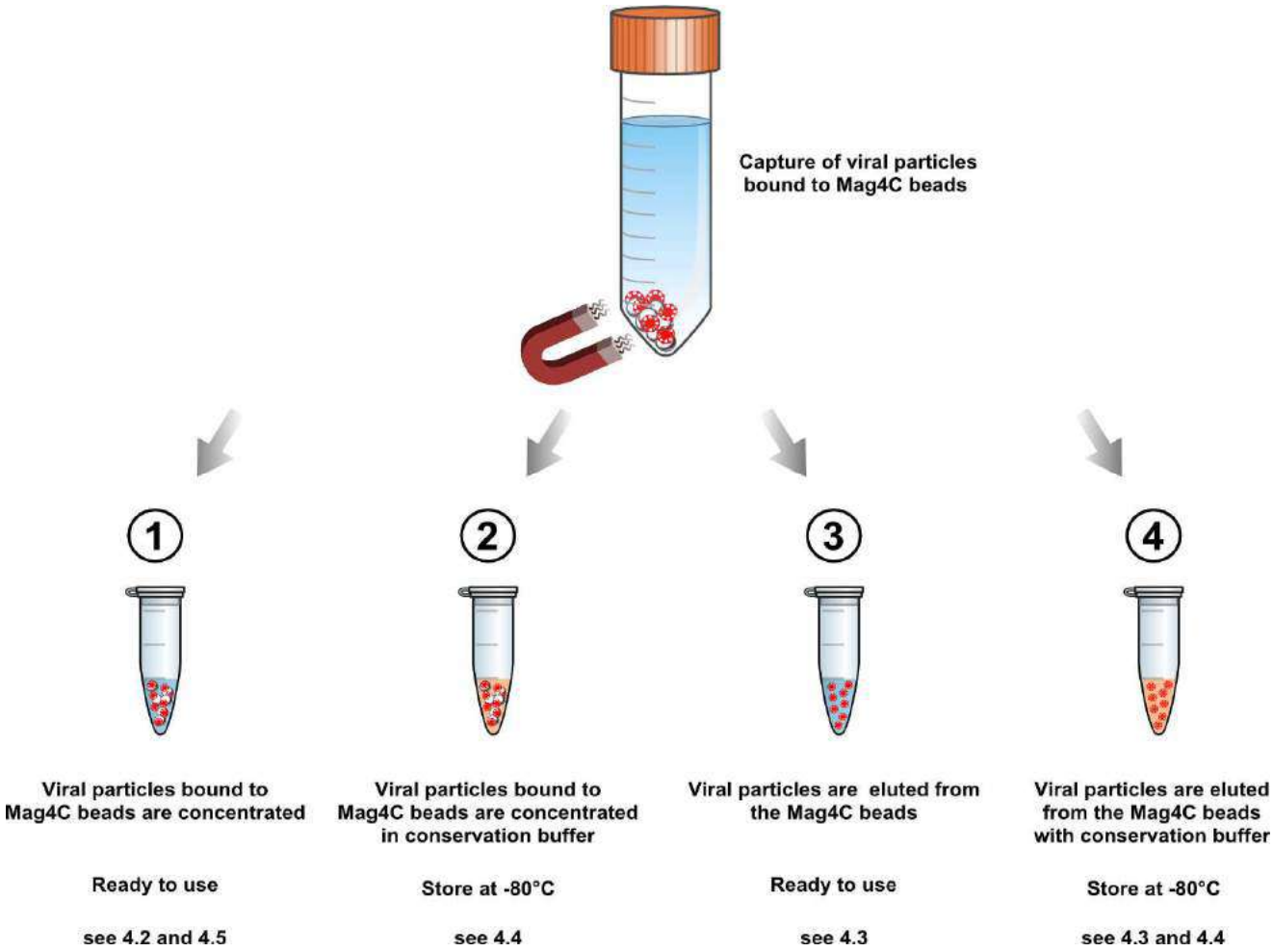
OPTIONAL: A washing procedure can be performed after this step:

i. Keep the tube on the Magnetic Separation Rack and slowly add PBS (same volume as the initial medium).
ii. Incubate 5 min on the Magnetic Separation Rack.
iii. Discard the supernatant.
iv. Proceed to step 4

Table 2 Recommended incubation time on the Magnetic Separation Rack according to the volume

Tube volume	Time on Magnetic Separation Rack
1 mL	15 min
10 mL	20 min
50 mL	30 min

4) The virus/Mag4C-Lv beads complexes can be used according to the following 4 options



(1) viruses are concentrated with Mag4C-Lv beads into smaller volumes of PBS (with Ca^{2+} and Mg^{2+}) or complete cell culture medium and used immediately for assay (for example see 4.5). Determine the appropriate volume of PBS/medium to add according to the expected final concentration.

(2) viruses are concentrated with Mag4C-Lv beads into smaller volumes of Conservation Buffer for long term storage (see 4.4)

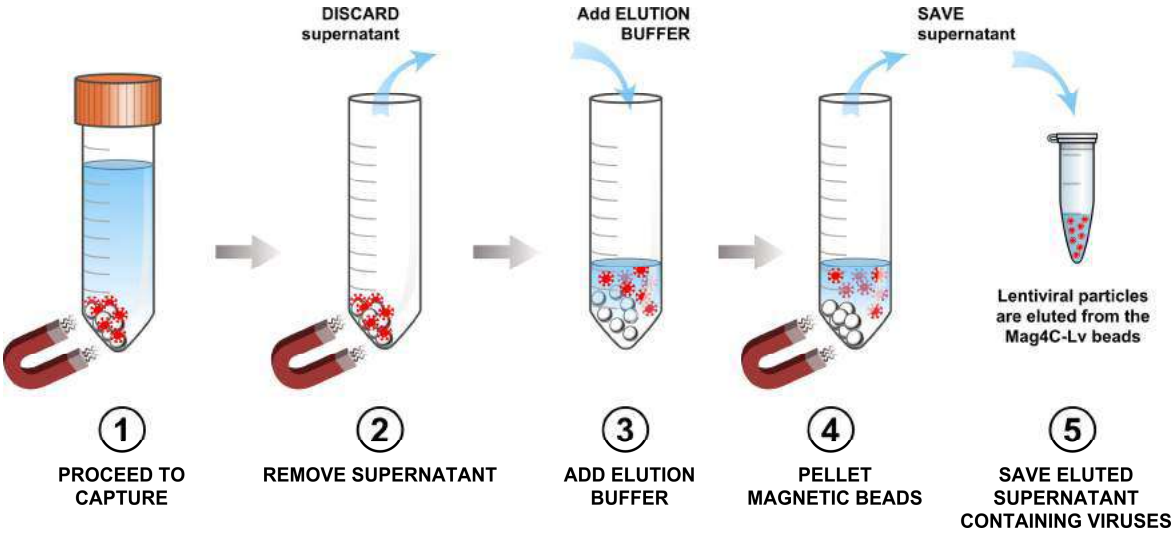
(3) viruses are eluted from Mag4C-Lv beads (see 4.3), concentrated into smaller volumes of Elution Buffer and used immediately for assay

(4) viruses are eluted from Mag4C-Lv beads (see 4.3), concentrated into smaller volumes of Elution Buffer plus Conservation Buffer for long term storage (see 4.4)

Keeping the Mag4C-Lv beads bound to viruses offers several advantages especially in terms on *in vitro* and *in vivo* infectivity as it allows using the Magnetofection™ technology (see 4.5):

4.3. Elution Procedure

This step is optional. You can choose to keep the nanobeads associated to the virus or remove them and have a "beads-free" concentrated virus. The **Elution Buffer** is a ready-to-use buffer, specifically designed to elute lentiviruses bound to the Mag4C-Lv nanobeads without impairing their infectious properties.



Important Note: For storage of **eluted** viruses, go directly to the section 4.4

1) Proceed to capture the virus as previously described and discard the supernatant (see 4.2 – steps 1 to 3).

Add the Elution Buffer to the virus/Mag4C-Lv beads complexes. Determine the appropriate volume of Elution Buffer to add according to the expected final concentration (see Table 3). For example, if the initial virus solution is 1mL and you want to concentrate 10 fold, then add 100µL of Elution Buffer. use volumes of elution buffer recommended to concentrate 10X and adjust to initial volume with PBS or culture medium so as not to concentrate viral particles.

Table 3 Volume of Elution Buffer for concentration and immediate use

Starting viral solution	Expected concentration 100X	Expected concentration 50X	Expected concentration 10X	Washing or medium exchange
1 mL	10 µL	20 µL	100 µL	1 mL
5 mL	50 µL	100 µL	500 µL	5 mL
10 mL	100 µL	200 µL	1 mL	10 mL
50 mL	500 µL	1 mL	5 mL	50 mL

2) After addition of the Elution Buffer, incubate for 5 to 10 min at RT.

3) Place the tube on the Magnetic Separation Rack and incubate 10 to 30 min at RT.

NOTE: Adjust incubation time on the Magnetic Separation Rack according to the volume (refer to table 2).

4) Save the supernatant containing viruses and discard pellet of Mag4C-Lv nanobeads.

5) The concentrated viruses solution can be used for downstream assay or proceed to section 4.4 for storage.

4.4. Conservation Procedure

Conservation procedure with Mag4c-Lv nanobeads.
Conservation buffer is 100% compatible with Mag4C-Lv nanobeads. In this way, Conservation Buffer can be added right after the capture procedure. Conservation Buffer allows virus storage for several months at -80°C and preservation of the virus titer upon freeze/thaw cycles.

Conservation Buffer preparation: Dilute 1 volume of the Conservation Buffer (5x) in 4 volumes of PBS (1X final). For 100 µL conservation buffer, add 20 µL of buffer to 80 µL of PBS.

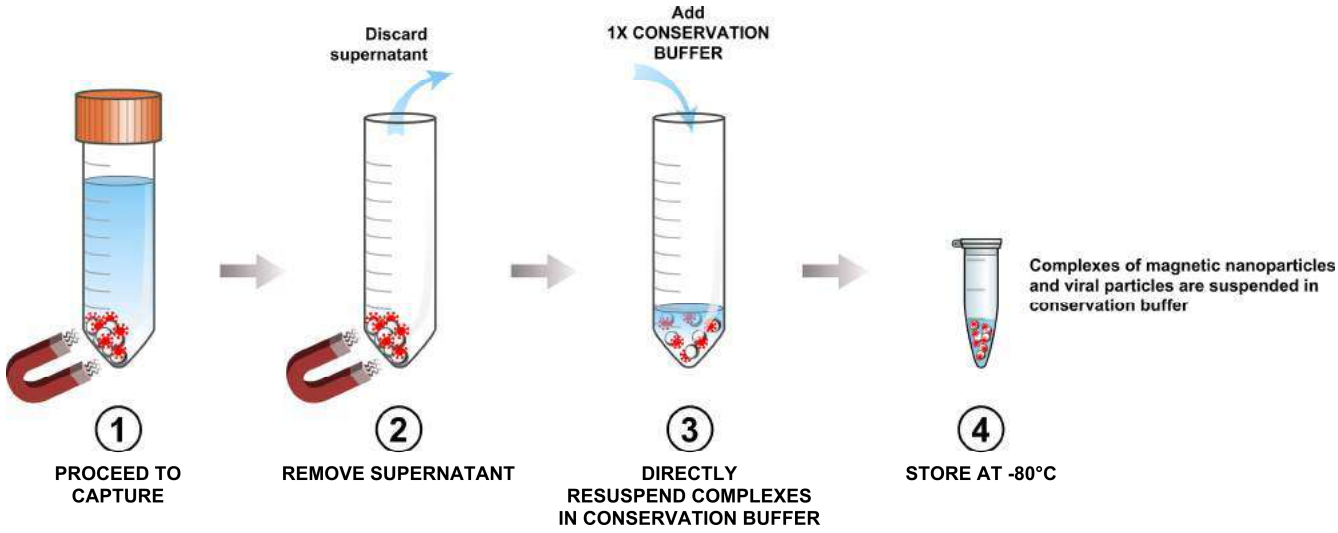
1) Proceed to capture the virus as previously described and discard the supernatants (see 4.2 – steps 1 to 3)

2) Remove the tube form the Magnetic Separation Device

3) Add freshly prepared conservation buffer to the complexes. To concentrate the virus solution, use smaller volume of buffer.

4) Store the complexes at -80°C.

NOTE: To reduce freezing/thawing cycles, it is recommended to aliquot virus for long term storage.



Conservation procedure after elution.
Conservation Buffer can be added right after the elution step. Conservation Buffer allows virus storage for several months at -80°C and preservation of the virus titer upon freeze/thaw cycles.

1) Proceed to capture the virus as previously described and discard the supernatants (see 4.2 – steps 1 to 3)

2) Add the Elution Buffer to the virus/Mag4C-Lv beads complexes (see Table 4 for Elution Buffer volume).

Table 4 Volume of Elution Buffer (EB) and Conservation Buffer (CB) for concentration and storage

Starting viral solution	Expected concentration 100X		Expected concentration 50X		Expected concentration 10X		Washing or medium exchange	
	EB	CB	EB	CB	EB	CB	EB	CB
1 mL	8 µL	2 µL	16 µL	4 µL	80 µL	20 µL	800 µL	200 µL
5 mL	40 µL	10 µL	80 µL	20 µL	400 µL	100 µL	4 mL	1 mL
10 mL	80 µL	20 µL	160 µL	40 µL	800 µL	200 µL	8 mL	2 mL
50 mL	400 µL	100 µL	800 µL	200 µL	4 mL	1 mL	40 mL	10 mL

3) After addition of the Elution Buffer, incubate for 5 to 10 min at RT.

4) Place the tube on the Magnetic Separation Rack and incubate 10 to 30 min at RT.

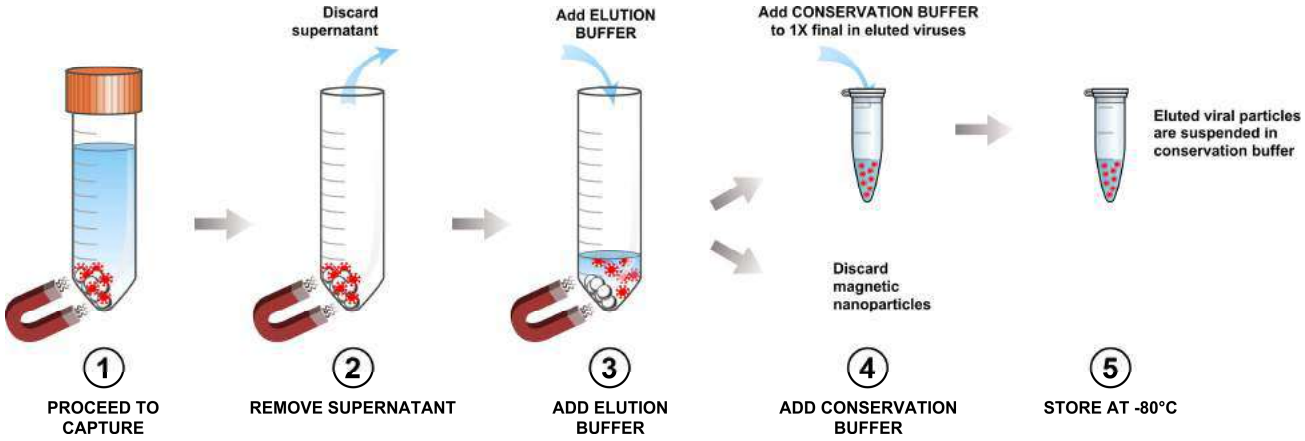
NOTE: Adjust incubation time on the Magnetic Separation Rack according to the volume (refer to table 2).

5) Save the supernatant containing viruses and discard pellet of Mag4C-Lv nanobeads.

6) Add the Conservation Buffer (5x) directly to the eluted viruses solution for a 1X final concentration. See Table 4.

7) Store virus at -80°C

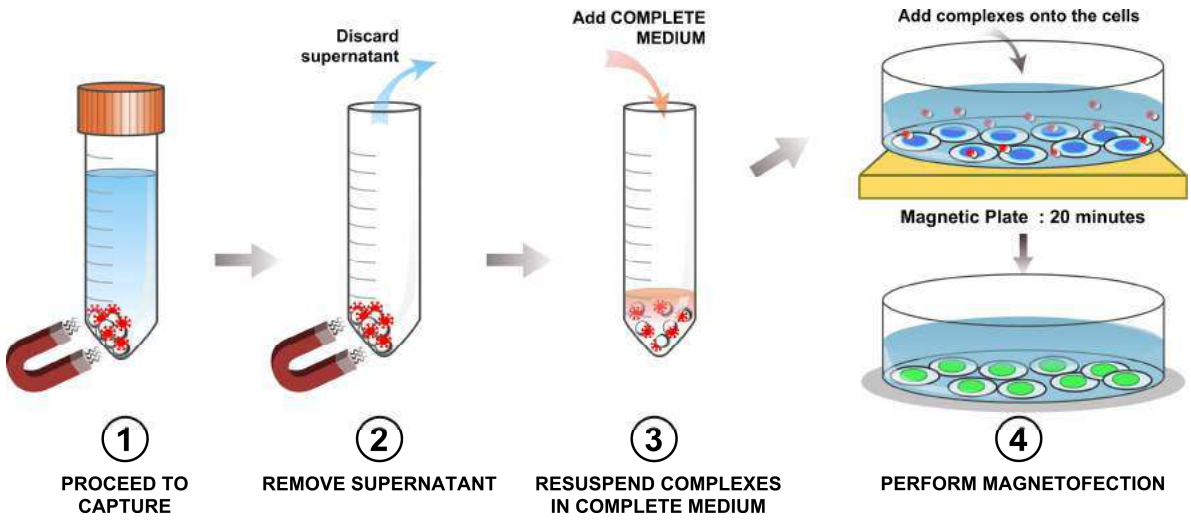
NOTE: To reduce freezing/thawing cycles, it is recommended to aliquot virus for long term storage.



4.5. Magnetofection™ Procedure

Keeping the Mag4C-Lv beads bound to viruses offers the Magnetofection™ technology advantages. This method allows concentrating the entire viral dose on the cells quickly, accelerating the transduction process and infecting non-permissive cells. The virus infection efficiency is considerably increased and virus adsorption can be synchronized without modification of the virus. Targeted/confined transduction to specific area (magnetic targeting) can also be accomplished.

After capture, concentration and storage (with the nanobeads), the Magnetofection procedure can be performed for *in vitro* transduction or *in vivo* infection. Specific protocols are available directly on our website for *in vitro* and *in vivo* experiments.



Magnetofection *in vitro* on adherent cells

1) Perform the capture and concentration of the virus as described above (see 4.2, steps 1-4) or thaw the virus/Mag4C-Lv beads complexes vial (4.4 conservation with the beads).

2) This protocol is given for a 24-well plate format; refer to the Magnetofection protocol for other sizes of culture dishes.

3) Plate the cells the day prior transduction. Best results are achieved if cells are at least 60-80 % confluent at the time of Magnetofection (if required refer to the suggested cell number in the Magnetofection protocol).

4) Add the virus/Mag4C-Lv beads complexes to the cells at the desired MOI in a drop wise manner.

5) Mix by gently rocking the plate to ensure correct dispersion of magnetic complexes within culture medium.

6) Place the cells upon the specific magnetic plate (see section 3) for 20-30 minutes.

NOTE: Optionally after this incubation, a medium change can be performed while maintaining the magnetic plate under the cell culture.

7) Remove the magnetic plate and cultivate the cells under standard conditions until evaluation of the transduction experiment.

NOTE: Optionally a medium change can be performed after 24 hours.

- For suspension cells, please refer to the Magnetofection protocol (ViroMag and ViroMag R/L or Viro-MICST)
- For *in vivo*, please refer to the *in vivo* Magnetofection protocol

5. Appendix

5.1. Critical Parameters for best performance

1) Mag4C-Lv quantity. We usually observe good capture efficiency with 20µL of Mag4C-Lv beads for 10^6 infectious viral particles. However the efficiency may depend on the virus type used and the medium composition. Consequently, we suggest you to start by testing a range of Mag4C-Lv volumes in order to obtain the best experimental conditions.

2) Magnetic Separation. The capture, concentration and elution on the Magnetic Separation Rack depend on the amount/concentration of virus used. Indeed, longer incubation under the magnetic field might be required with very low viral titers whereas with high viral dose short incubation times are sufficient.

3) Elution Buffer. The volume of Elution Buffer as well as the incubation time for the elution might have to be adjusted depending on the virus concentration and the amount of Mag4C-Lv beads.

4) Conservation Buffer. Conservation buffer preserves lentivirus effect after -80°C storage with multiple freezing/thawing cycles. However, we recommend to aliquot viral particles solution in conservation buffer to avoid virus degradation and thus ensuring the best conservation conditions.

5.2. Optimization

In order to get the best out of **Mag4C-Lv Kit**, several parameters can be optimized.
OZ Biosciences team has investigated numerous factors during the course of the R&D program. Based on our experience, we recommend you to start from the experimental procedures described above (section 4) and optimize one parameter at a time

1) Start by optimizing the Mag4C-Lv beads dose. To this end, vary the amount of Mag4C-Lv in the range suggested in the Table 1. For instance, for 10^6 viral infectious particles or for viral preparation ≤ 2mL use 10 to 40 µL of Mag4C-Lv. After the capture step, you can test the supernatant for remaining infectivity. If capture is efficient, the supernatant should not be infectious.

2) Depending on the quantity of virus the time of incubation for the complexes formation as well as for the capture on the Magnetic Separation Rack should be extended.

3) If using a complex medium (organic fluid...), we recommend diluting sample with PBS at least 1.5 times. Do not dilute medium excessively to avoid large dispersion of viral particles that hamper efficient virus capture.

5.3. "Troubleshooting"

Problems	Comments and Suggestions
Low capture and concentration efficiency.	1. **Virus titer.** Ensure that virus titer used corresponds to infectious viral particles and not physical particles. A 1 to 1000 ratio can be found between physical and infectious particles. 2. **Virus quality.** Even though Mag4C-Lv has been designed to work with culture medium containing viral particles, for the best efficiency viruses should be as pure as possible, exempt of contaminants 3. **Medium Composition.** Use higher volume of Mag4C-Lv beads to raise the capture efficiency. If too viscous, medium can be diluted with PBS (with Ca^{2+} and Mg^{2+}) or with salt balanced physiological buffer at least 1.5 times. 4. **Capture time.** 15 min should lead to an optimal capture. Still it may be necessary to incubate Mag4C-Lv beads longer with virus to ensure complete capture. 5. **Medium purity.** Be sure that no contaminants are present in medium. Endotoxins or microorganisms will easily bind on beads and compete with virus fixation. 6. **Reagents temperature.** Reagents should have an ambient temperature and be vortexed prior to use
Low elution efficiency	1. **Incubation time.** 10 min in presence of Elution Buffer and 30 min on separation rack should lead to optimal elution. It may be necessary to increase both incubation periods to 20 min and 45 min respectively. 2. **Temperature.** Perform the elution process at 37°C. 3. **Washing.** Before elution procedure, wash magnetic complexes with PBS as suggested in paragraph 4.2.
Low infection efficiency with or without Magnetofection	1. **Cell density.** A non-optimal density can lead to poor efficiency. The optimal confluency should range from 50 to 90% (true confluency, corresponding to 90% visual confluency) but most favorable density may vary according to the cell type. 2. **Type of virus.** Ensure that the virus can infect (being expressed) the cells. Another viral-driven promoter can be used as a control. 3. **Cell condition.** Use freshly thawed cells that have been passaged at least once. Cells should be healthy and assayed during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) can alter the transduction efficiency. 4. **Incubation time and transduction volume.** 1) The optimal time range between transduction and assay varies with cells, promoter, expression product, etc... The transduction efficiency can be monitored after 24 – 96h by analyzing the gene product. Several reporter genes can be used to quantitatively monitor gene

	expression kinetics. 2) To increase transduction efficiency, transfection volume can be reduced for the first 24 hours. 5. **Transgene detection assay.** Ensure that your post-transduction assay is properly set up and includes a positive control. 6. **Multiplicity of Infection (MOI).** Be sure that the MOI is properly calculated. There could be a 1/10 to 1/1000 ratio between physical and infectious particles. 7. **shRNA design.** The design of an efficient shRNA is crucial. Ensure to use a validated shRNA sequence encoded in the expression vector.
Cellular toxicity	1. **Unhealthy cells.** 1) Check cells for contamination, 2) Use new batch of cells, 3) Ensure culture medium condition (pH, type of medium used, contamination etc...), 4) Cells are too confluent or cell density is too low, 5) Verify equipments and materials. 2. **Infection is toxic.** Most of the lentiviruses used are not replicative. Be sure that the cell line doesn't express the missing region for replication. It should be noted that even if lentivirus can't replicate into the cells, it can still express viral proteins that can be toxic and cause cytopathic effect. Oncolytic viruses kill cells. 3. **Concentration of Mag4C-Lv/virus complexes too high.** Decrease the amount of complexes added to the cells by lowering the MOI. 4. **Incubation time.** Reduce the incubation time of complexes with the cells by replacing the transfection medium by fresh medium after 4 h to 24 h. 5. **Key gene silencing.** If the targeted gene is essential for cell survival or if a key gene is non-specifically silenced by the si- or shRNA, this can lead to cell death.

5.4. Quality Controls

To assure the performance of each batch of **Mag4C-Lv Kit** produced, we qualify each lot using rigorous standards. *In vitro* assays are conducted to qualify the quality and activity of each kit component.

Components	Standard Quality Controls
Mag4C-Lv & buffers	1. Sterility. Thioglycolate assay: absence of fungal and bacterial contamination shall be obtained for 14 days.
Mag4C-Lv & buffers	2. Quality and size homogeneity of the magnetic nanoparticles. 3. Stability of the magnetic nanoparticles formulations. 4. Mag4C-Lv capture and transduction efficacies with a recombinant lentivirus on NIH-3T3 or HeLa cells. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot. 5. Elution and preservation performance
Magnetic Separation Rack	6. Tests of solidity and Test of the magnetic field force

6. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate *(standard size for all cell culture support)*
Mega Magnetic plate *(mega size to hold 4 culture dishes at one time)*
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
Magnetofectamine™ (*for all nucleic acids*)
NeuroMag *(dedicated for neurons)*
SilenceMag *(for siRNA application)*
Transfection enhancer:
CombiMag *(to improve any transfection reagent efficiency)*
Viral Transduction enhancers:
ViroMag R/L *(specific for Retrovirus and Lentivirus)*
AdenoMag *(for Adenoviruses)*
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
VeroFect *(for Vero cells)*
FlyFectin *(for Insect cells)*
i-MICST TECHNOLOGY
Viro-MICST (*to transduce directly on magnetic cell purification columns)*
3D TRANSFECTION TECHNOLOGY
3Dfect *(for scaffolds culture)* / 3DfectIN *(for hydrogels culture)*
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit (*for **H**igh **Y**ield **P**rotein **E**xpression)*
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN *(delivery reagent for antibodies)*
Pro-DeliverIN *(delivery reagent for protein in vivo and in vitro)*
PLASMIDS PVECTOZ
pVectOZ-LacZ / pVectOZ-SEAP / pVectOZ-GFP / pVectOZ-Luciferase
ASSAY KITS
Bradford – Protein Assay Kit
MTT cell proliferation kit
β-Galactosidase assay kits (CPRG/ONPG)
BIOCHEMICALS
D-Luciferin, K^+ and Na^+ 1g
X-Gal powder 1g / G-418, Sulfate 1g

Purchaser Notification

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